Author's response to reviews

Title: Reduced expression of ezrin in urothelial bladder cancer signifies more advanced tumours and an impaired survival: validatory study of two independent patient cohorts

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Version: 2
Date: 3 April 2014

Author's response to reviews: see over
Dear Editor,

Thank you for your positive response and invitation to resubmit our paper “Reduced expression of ezrin in urothelial bladder cancer signifies clinically advanced disease, tumour progression and an impaired survival: validator study of two independent patient cohorts” by Gustav Andersson et al. We are grateful for helpful and constructive comments and suggestions from the Reviewers. A further revised manuscript has been prepared taking them into account, as outlined in the point-by-point response below, and highlighted in yellow in the revised manuscript. We have also taken the opportunity to check the manuscript for typos and inconsistencies.

Please note that we have shortened the title, on our own initiative, in order to make it more comprehensive. We believe that, since there was no association between ezrin expression and time to progression, it may be confusing to state an association with progression in the title (actually meaning a more advanced T-stage). The revised title is now: “Reduced expression of ezrin in urothelial bladder cancer signifies more advanced tumours and an impaired survival: validator study of two independent patient cohorts”. We hope you agree with us that this title is more appropriate.

With these improvements of the manuscript we hope you will find our paper suitable for publication in BMC Urology.

Yours sincerely,

Karin Jirström
Reviewer's report

Title: Reduced expression of ezrin in urothelial bladder cancer signifies clinically advanced disease, tumour progression and an impaired survival: validatory study of two independent patient cohorts

Version: 1
Date: 4 January 2014
Reviewer: Caj Haglund
Reviewer's report:
The subject of the study is interesting and clinically relevant. The materials are quite large and well characterised. Ezrin has been previously studied in bladder cancer, but only in two reports. Further validation of the results are therefore needed. The association between ezrin and podocalyxin is particularly interesting.
I recommend the paper to be accepted, but some changes are needed before the manuscript is ready for publication:

1. Please make it more clear, that membranous and cytoplasmic staining of ezrin were analysed separately, since in many papers staining intensity and percentage of positive cells have been combined. Did the authors try to combine membranous and cytoplasmic staining?
   Response: Since there was no correlation between membranous and cytoplasmic ezrin expression (see also p 4 below) and the latter was not prognostic, a combined score was, as expected, not of additional prognostic value. This has now been denoted in the Results section, p 10.

2. The mean value of two spots were used. Since low expression represents more malignant behaviour, one could also consider the lower score value to be more representative of the most aggressive cell population. This should be discussed.
   Response: This is a valid point. There was however no obvious heterogeneity in membranous or cytoplasmic ezrin staining between duplicate cores. The following clarifications have now been made:
   1. The sentence "there was no obvious heterogeneity regarding membranous or cytoplasmic ezrin expression between duplicate cores” has now been added to the Results section, p 8.
   2. The sentence “Since the percentage of membranous staining was similar between duplicate cores, use of best or worst score did, as expected, not improve the prognostic value of ezrin expression (data not shown).” has now been added to the Results section, p 10.

3. Interpretation of markers whose expression decreases in malignancies is always difficult, since most IHC samples include negative areas, that can be negative for technical reasons. When evaluating the cytoplasmic intensity of ezrin, the authors decided to choose the most intense area. If loss ezrin is a marker for malignant behaviour this might not be biologically the right choice? Please discuss this question.
   Response: First of all, we apologize for having described the annotation of cytoplasmic staining in a confusing way, and 10% is not the correct limit for staining evaluation, we used the dominating intensity (>50%). The Material and Methods section has therefore been corrected on p 7 as follows: " When evaluating cytoplasmic staining the dominating intensity for each core was determined. A mean value of the two samples from each tumour was used
4. Was there any correlation between membranous ezrin and cytoplasmic ezrin?
Response: No there was no significant correlation. This has now been denoted in the Results section page 8.
5. There are some careless mistakes in affiliations.
Response: Thank you, this has now been corrected.

Level of interest: An article of importance in its field
Quality of written English: Needs some language corrections before being published
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests: No

Reviewer's report
Title: Reduced expression of ezrin in urothelial bladder cancer signifies clinically advanced disease, tumour progression and an impaired survival: validatory study of two independent patient cohorts
Version: 1 Date: 12 March 2014
Reviewer: fadi brimo
Reviewer's report:

MAJOR REVISIONS:
Interesting study but the lack of clear data about the cohort and the use of survival solely as an end point is very confusing..it should be clarified
1) are all cases from TURB? or cystectomy as well?
Response: All cases are from TURB specimens, this has now been clarified for each cohort, respectively, on pp 5 and 6, Material and Methods section. Please note that we did indeed at time to progression in pTa and pT1 tumours according to ezrin expression, but foud no significant association. This has been denoted in the Results section p 10, and discussed on p 12.

2) TA includes low grade and high grade non invasive tumors?
Response: Yes.

3) In real practice invasive low grade urothelial carcinoma is very rare..almost all invasive tumors are high grade by definition..how can the authors explain the relatively high number of invasive low grade tumors in their cohort?
Response: This is a valid point, and while we cannot readily explain this, the viewpoint that invasive tumours per definition should be considered high grade is probably quite accurate from a clinical perspective. Again, given the fragmented character of many TURB-specimens, where T-stage is often more difficult to determine than grade, it is of great importance to identify additional biomarkers to help distinguish the biologically more aggressive tumours from the more indolent.

4) If we are dealing with TURBs, the three end points should be recurrence, progression, and death, not only death..lumping TA with T2 and correlating with death is confusing and does not account for the different treatment modalities.
that are used for those tumors. What I would suggest is to divide cases according to type of specimen (turb vs cystectomy) and present the results with three end points as suggested above for TURB specimens (recurrence, progression and survival). Cystectomy specimens should be correlated with recurrence and death.

Response: Since all specimens were TURB specimens, which has now been clarified, we believe that this issue should be solved. Stage has been included in the adjusted analysis. Moreover, we did look at preogression, see also response to p 1 above. There was no association between reduced ezrin expression and a more frequent recurrence rate. This has now been denoted on p 10, Results section.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:
NO CONFLICT OF INTEREST

Reviewer's report
Title: Reduced expression of ezrin in urothelial bladder cancer signifies clinically advanced disease, tumour progression and an impaired survival: validatory study of two independent patient cohorts
Version: 1 Date: 24 March 2014
Reviewer: Jose Mansure

Reviewer's report:

Major compulsory revisions:

1. The questions posed by the authors are well defined, but the authors have not provided sufficient analytical evidence of subject importance.
Response: We have previously published several biomarker studies with similar measures of effect in both cohorts. As regards ezrin expression, our findings are similar in two cohorts and also validate findings from previous studies.

2. The analysis does not record competing events (e.g. death from other causes) prior to disease specific death. A competing risk regression should be considered.
Response: With due respect, we have used both disease-specific death and death from overall causes in the analyses.

3. In table 1, the number of patients and their respective percentages should be presented for each clinicopathological factor. This will help the readers to evaluate factor distribution in each cohort.
Response: This information has now been added to Table 1

4. All of the K-M figures must include a "risk-table" showing the number of patients at risk during each time span.
Response: Life tables have now been added to all panels in Figure 3.
5. The methodology of Classification and Regression Tree (CRT) analysis, which has been used to assess the optimal prognostic cut-offs for ezrin expression, should have a more elaborate description.
Response: We have now added the original reference which describes the methodology. We have also stated that the same cutoffs were obtained by receiver operating characteristics ROC curve analysis (data not shown) in the Results section, p 9.

6. Furthermore, interpretations (discussion and conclusion) made by the authors seem unbalanced and inadequately supported by the data. For instance, the authors conclude that reduced membranous ezrin is associated with reduced survival. However, when the multivariate analysis was performed, this association was only marginally retained as an independent prognostic value for 5 year OS in the smaller cohort. It seems unlikely that the lack of an independent prognostic value of ezrin expression in the larger cohort is due to its strong link to tumor grade and stage as the distribution of tumor stage and grade in both cohorts are not significantly different [Reference 22-23] to fully support the hypothesis stated by the authors in the second paragraph of the discussion.
Response: The paragraph in the Discussion section, p 11 has now been rewritten as follows: ”Of note, ezrin expression only retained an independent prognostic value for 5-year OS in the smaller cohort, and neither for 5-year OS nor for DSS in the larger, clinically more well-characterized, cohort. Therefore, further validation of the prognostic value of ezrin expression in additional patient cohorts is warranted.”

Level of interest: An article of limited interest
Quality of written English: Acceptable
Statistical review: Yes, and I have assessed the statistics in my report.
Declaration of competing interests:
I declare that I have no competing interests